PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Di Rienzo et al.

Serial No.: 09/251,274

Filed: February 16, 1999

For: METHODS FOR DETECTION OF PROMOTOR POLYMORPHISM IN A UGT GENE PROMOTOR

Group Art Unit: 1655

Examiner: A. Chakrabarti

Atty. Dkt. No.: ARCD:357US/DLP

CERTIFICATE OF MAILING
37 C.F.R. 1.8

I hereby certify that this correspondence is being deposited with the U.S. Postal Service as First Class Mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231 on the date below:

10/10/00
DATE

David L. Parker

DECLARATION UNDER RULE 131

Anna Di Rienza, Lalitha Iyer and Mark Ratain hereby declare as follows:

1. We are joint inventors of the subject matter disclosed and claimed in the above referenced patent application.

2. We understand that the U.S. Patent and Trademark Office Examiner handling the referenced application has taken the position that the Beutler et al. article appearing in Volume 95 of the Proceedings of the National Academy of Science, USA, pp. 8170-8174, July 1998, is relevant to the patentability of the referenced invention.

3. While we do not agree with the Examiner's conclusion in this regard, particularly with respect to certain aspects of our invention as set forth in the claims, we would point out that we made the subject invention, or at least as much of the subject invention as may be shown in the Beutler et al. article, in this country prior to July 1998. This is evidenced by the enclosed Abstract of our work from the March 30 - April 1, 1998 meeting of the American Society for Clinical Pharmacology and Therapeutics held in New Orleans, Louisiana. This Abstract is attached as Exhibit "A" hereto, and we refer you in particular to Abstract OII-B-3.

4. As can be seen from this Abstract, we discovered the existence and relevance of the two new alleles of the UGT promotor, the 5 [$(TA)_5$ TAA] and 8 [$(TA)_8$ TAA] alleles, and discovered that these promotor alleles appeared to be unique to populations of African origin. We further demonstrate that in contrast to previously identified other polymorphic alleles, allele five carriers (genotypes 5/6 and 5/7) showed higher levels of glucuronidation of bilirubin and SN 38, and that these results suggested the allele five resulted in an increase in UGT gene expression.

5. The data referred to in this Abstract was presented at the American Society for Clinical Pharmacology and Therapeutics meeting on March 30 - April 1, 1998 in New Orleans, Louisiana, and the Abstract reflecting this work was published prior to that time.

6. WE HEREBY DECLARE THAT ALL STATEMENTS MADE OF OUR OWN KNOWLEDGE ARE TRUE AND THAT ALL STATEMENTS MADE ON INFORMATION AND BELIEF ARE BELIEVED TO BE TRUE; AND FURTHER THAT THESE STATEMENTS WERE MADE WITH THE KNOWLEDGE THAT WILLFUL FALSE STATEMENTS AND THE LIKE SO MADE ARE PUNISHABLE BY FINE OR IMPRISONMENT, OR BOTH, UNDER SECTION 1001 OF TITLE 18 OF THE UNITED STATES CODE AND THAT SUCH WILLFUL FALSE STATEMENTS MAY JEOPARDIZE THE VALIDITY OF THE APPLICATION OR ANY PATENT ISSUED THEREON.

Anna Di Rienzo — 10-2-00
Anna Di Rienzo — Date

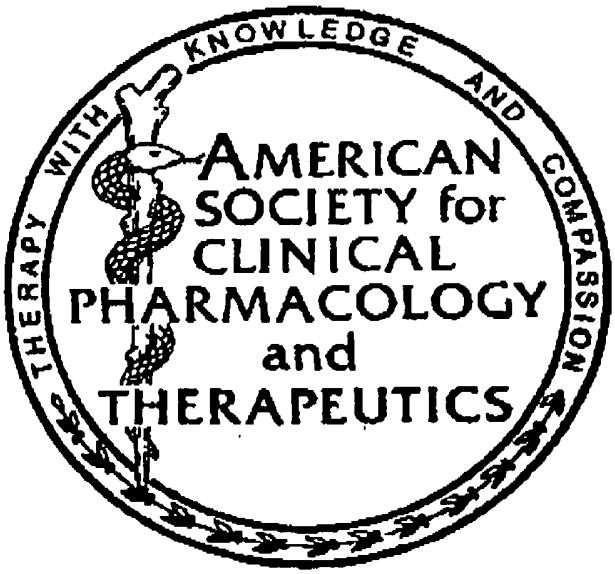
Lalitha Iyer — 10/2/00
Lalitha Iyer — Date

Mark J. Ratain — 10/2/00
Mark J. Ratain — Date

EXHIBIT "A"

NINETY-NINTH ANNUAL MEETING

ABSTRACTS of PAPERS



March 30 – April 1, 1998

The New Orleans Marriott

New Orleans, Louisiana

OII-B-2

GENETIC POLYMORPHISMS IN THE NAD(P)H: QUINONE OXIDOREDUCTASE (NQO1) GENE LOCUS. A Gaedigk, PhD[1], R.P. Tyndale, PhD[1,2], M. Jurima-Romet, PhD[3,4], EM Sellers[1], MD, PhD, J.S. Leeder, PharmD, PhD, Sect of Ped Clin Pharmacol, Children's Mercy Hospital, Kansas City, MO, [1]Dept of Pharmacol, Univ of Toronto, ON, [2]Bureau of Biologics and Radiopharmaceuticals, Health Canada, Ottawa, ON.

NQO1 catalyzes the two-electron reduction of quinone compounds and is involved in the reductive bioactivation of cytotoxic antitumor quinones such as mitomycin C. Three alleles with amino acid substitutions at positions 139 and 187 have been identified: the functional Arg139/Pro187 allele which we termed NQO*1; the non-functional allele Arg139/Ser187 (NQO*2) and the Trp139/Pro187 allele (NQO*3), which is associated with a diminished activity. Interethnic variability in allele frequencies has not been studied. We developed PCR-based genotyping assays and determined NQO1 allele frequencies in Caucasian, Canadian Native Indian, Canadian Inuit and Chinese populations as shown:

population	n=	*1 allele	*2 allele	*3 allele
Chinese	86	0.47	0.49	0.04
Inuit	93	0.54	0.46	none
Native Indian	110	0.59	0.40	0.009
Caucasian	282	0.79	0.17	0.04

The non-functional *2 allele was more frequent in Asian populations [Chinese vs Caucasian p <0.0001] and therefore, a greater proportion of individuals may lack NQO1 activity. These subjects may exhibit resistance to quinone-based cancer therapy due to a decreased production of cytotoxic drug metabolites.

OII-B-3

TWO NEW ALLELES IN THE PROMOTER OF THE BILIRUBIN UDP-GLUCURONOSYL TRANSFERASE 1 (UGT1A1) GENE. A DiRienzo* PhD, D. Hall*, BS, L. Iyer, PhD, and M.J. Ratain, MD. Center for Medical Genetics and Committee on Clinical Pharmacology, Dept. of Medicine, University of Chicago, Chicago, IL.

Variation in the $(TA)_6TAA$ element in the promoter of the UGT1A1 gene was previously associated with Gilbert's syndrome, a chronic, mild, unconjugated hyperbilirubinemia. Allele 7 $[(TA)_7TAA]$ was shown to result in reduced levels of gene expression and increased serum bilirubin compared to allele 6 $[(TA)_6TAA]$. We have also shown that this allele results in reduced in vitro glucuronidation of bilirubin and SN-38 — the active metabolite of irinotecan (CPT-11) (see Iyer et al, this meeting). Here, we surveyed population variation at the $(TA)_6TAA$ element in different ethnic groups and observed frequencies for allele 7 which varied between 25% and 47%. We also found two new alleles, 5 $[(TA)_5TAA]$ and 8 $[(TA)_8TAA]$, which appear to be unique to populations of African origin: the two alleles have frequencies of 5% and 6%, respectively. In African Americans, Bilirubin and SN-38 glucuronidation assays in liver microsomes of two allele 5 carriers (genotypes 5/6 and 5/7) showed high levels, beyond the confidence intervals for the 6/6, 6/7 and 7/7 genotypes. These results suggest that allele 5 may result in increased UGT1A1 gene expression. These new alleles indicate that up to 10 genotypes may exist at the $(TA)_6TAA$ element probably resulting in different phenotypes with regard to bilirubin conjugation and pharmacokinetics of CPT-11.

OII-B-4

DETERMINATION OF HUMAN FMO ACTIVITY USING CAFFEINE METABOLISM AND IDENTIFICATION OF FMO MUTANTS. [1]C.S. Park, PhD, [1]W.G. Chung, PhD, [1]J.H. Kang, MD, [1]K.H. Lee, MD, [2]H.K. Roh, MD, and [1]Y.N. Cha, PhD, [1]Dept. of Pharmacol., [2]Dept. of Int. Med., Inha Univ., Inchon, Korea.

It is known that the major form of flavin-containing monooxygenases (FMOs) present in adult human liver is FMO3. After finding that production of theophylline (TP) and theobromine (TB) from caffeine (CA) is catalyzed primarily by hepatic FMO (BBRC. 235: 645-648), we have attempted to phenotype human with respect to their FMO activity *in vivo* by administering coffee (110mg CA). Hr urine samples between 4 and 8hr were collected from 82 healthy Korean volunteers and their urinary CA metabolites were analyzed. The ratio of urinary (TB+TP)/(CA) was used as an index of FMO activity. Upon identifying the volunteers with high and low FMO activities, we have sequenced their FMO3 genes in full (9 exons and junction sequences between introns and exons) from their genomic DNA obtained from peripheral blood.

Comparing the sequences of FMO3 gene in subjects with high and low activities, we found 6 point mutation sites on exon sequences. In most subjects with low activity, we found point mutations on Glu→Lys[158] (GAG→AAG) and/or Glu→Gly[308] (GAG→GGG) in exons 4 and 7, respectively, using the restriction enzymes *Mlsf I* (for exon 4) and *Dra II* (for exon 7). A stop codon (Gly[168]→STOP; GGA→TGA) was found in a subject with extremely low FMO activity. Also homozygotes with mutations on the above two mutation sites showed low FMO activity. These subjects may have defective metabolism for many clinically used drugs and dietary plant alkaloids which are metabolized by FMO.

PIII-1

AMIODARONE ASSOCIATED INCREASE IN SERUM CREATINE IS NOT MEDIATED BY ALTERED CIMETIDINE SENSITIVE RENAL SECRETION. PT. Pollak, MD, PhD, Dept of Medicine, Dalhousie University, Halifax, Canada.

We previously reported a mean increase in serum creatinine (SCr) of 11% above baseline in a cohort of 28 patients receiving amiodarone (Am) for 12 mo. One potential mechanism for an increase in SCr of this magnitude would be suppression of proximal tubular secretion of SCr as seen with administration of cimetidine (Ci). We monitored a new group of 12 patients receiving Am for 18 mo. A mean increase of 14% in SCr stabilized at 9% by 18 mo. At this point 24 h creatinine clearances (CrCl) were performed in each patient with and without coadministration of Ci 400 mg QID. Median CrCl fell from 1.34 to 1.0 mL/s ($p<0.01$). This 25% drop in CrCl on Ci indicates Am did not block Ci sensitive SCr secretion. This study confirms Am causes a consistent, long-term increase in SCr, but the mechanism of action remains undetermined.